

Ser. No. 09/686,499
Reply to O/A of 8/27/2003

§ 1.136(a), and any fees required therefore are hereby authorized to be charged to our Deposit Account 20-0823.

Please amend the above-identified application as set forth below, and enter the following Remarks.

Listing of Claims:

This listing of claims replaces all prior versions and listings of claims in the application.

1. (previously amended) An attenuated derivative of a pathogenic microorganism which comprises:

(a) a non-functional native chromosomal essential gene;

(b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene; and

(c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product;

wherein said complementing gene of (b) is a functional replacement for said essential gene of (a), wherein the desired gene is stably maintained in a progeny population of the microorganism.

2. (original) The microorganism of claim 1, wherein the microorganism is a member of the *Enterobacteriaceae* and the extrachromosomal vector is a plasmid.

3. (previously amended) The microorganism of claim 2, further comprising an inactivating mutation in a gene selected from the group consisting of a *pab* gene, a *pur* gene, an *aro* gene, *nadA*, *pncB*, *galE*, *pmi*, *fur*, *rpsL*, *ompR*, *htrA*, *hemA*, *cdt*, *cya*, *crp*, *dam*, *phoP*, *phoQ*, *rfc*, *poxA*, *galU*, *mviA*, *sodC*, *recA*, *ssrA*, *sirA*, *inv*, *hilA*, *rpoE*, *flgM*, *tonB*, and *slyA*.

4. (original) The microorganism of claim 3, wherein the desired gene product is an antigen.

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5. (original) The microorganism of claim 4, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen, a gamete-specific antigen, an allergen, and a tumor antigen.
6. (original) The microorganism of claim 2, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapD*, *dapE*, *dapF*, and *asd*.
7. (previously amended) The microorganism of claim 6, wherein the non-functional native chromosomal essential gene is an *asd* gene wherein said *asd* gene comprises an insertion or a deletion.
8. (original) The microorganism of claim 2, wherein the recombinant complementing gene lacks an RNA polymerase -35 recognition sequence and a promoter -10 sequence.
9. (original) The microorganism of claim 8, wherein the recombinant complementing gene is an *asd* gene.
10. (original) The microorganism of claim 2, wherein the desired gene is operably linked to a eukaryotic promoter.
11. (previously amended) The microorganism of claim 10, wherein the eukaryotic promoter is a CMV (cytomegalovirus) promoter.
12. (previously amended) A recombinant vector comprising a recombinant complementing gene, wherein the recombinant complementing gene lacks an RNA polymerase -35 recognition sequence and a promoter -10 sequence,
wherein the recombinant complementing gene is a functional replacement for a non-functional native chromosomal essential gene when the vector is present in a microorganism having a non-functional native chromosomal essential gene.

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13. (previously amended) The recombinant vector of claim 12, wherein the vector is a plasmid capable of expressing the recombinant complementing gene in a microorganism that is a member of the *Enterobacteriaceae*.
14. (previously amended) The recombinant vector of claim 12, wherein the recombinant complementing gene encodes an enzyme that catalyzes a step in the biosynthesis of DAP (mesodiaminopimelic acid).
15. (previously amended) The recombinant vector of claim 14, wherein the recombinant complementing gene is an *asd* gene.
16. (previously amended) The recombinant vector of claim 12, further comprising a gene encoding a desired gene product.
17. (original) The recombinant vector of claim 16, wherein the desired gene product is an antigen.
18. (original) The recombinant vector of claim 17, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen, a gamete-specific antigen, an allergen, and a tumor antigen.
19. (original) The recombinant vector of claim 16, wherein the desired gene product is therapeutic to a vertebrate.
20. (original) The recombinant vector of claim 19, wherein the desired gene product is selected from the group consisting of a lymphokine, a cytokine, and a sperm-specific or egg-specific autoantigen.

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21. (original) The recombinant vector of claim 16, wherein the desired gene product is operably linked to a eukaryotic promoter.

22. (original) The recombinant vector of claim 21, wherein the eukaryotic promoter is a CMV promoter.

23-44. (canceled)

45. (previously added) An attenuated derivative of a pathogenic microorganism which comprises:

(a) a mutation of a polynucleotide sequence that renders a native chromosomal essential gene non-functional;

(b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene is a functional replacement for said essential gene of (a) and wherein said complementing gene can recombine to replace the essential gene of (a); and

(c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product;

wherein the desired gene is stably maintained in a progeny population of the microorganism.

46. (previously added) An attenuated derivative of a pathogenic microorganism which comprises:

(a) a non-functional native chromosomal essential gene;

(b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene can recombine to replace the non-functional chromosomal essential gene;

(c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product; and

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(d) an inactivating mutation in a native gene selected from the group consisting of a *pab* gene, a *pur* gene, and *aro* gene, *nadA*, *pncB*, *gale*, *pmi*, *fur*, *rpsL*, *ompR*, *htrA*, *hemA*, *cdt*, *cya*, *crp*, *dam*, *phoP*, *phoQ*, *rfc*, *poxA*, *falU*, *mviA*, *sodC*, *recA*, *ssrA*, *sirA*, *inv*, *hilA*, *rpoE*, *flgM*, *tonB*, and *slyA*;

wherein said complementing gene of (b) is a functional replacement for said essential gene of (a), wherein the desired gene is stably maintained in a progeny population of the microorganism.